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IS 13657 (1993): Aspartame, Food Grade [FAD 8: Food Additives]

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भारतीय मानक

एस्पार्टेम, खाद्य ग्रेड — विशिष्टि

Indian Standard

ASPARTAME, FOOD GRADE — SPECIFICATION

UDC 664·099·6 : 547·583·2

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BUREAU OF INDIAN STANDARDS
MANAK BHAVAN, 9 BAHADUR SHAH ZAFAR MARG
NEW DELHI 110002

July 1993

Price Group 2

Food Additives Sectional Committee, FAD 8

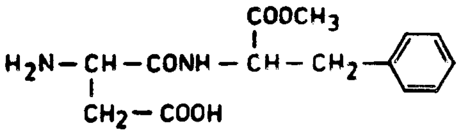
FOREWORD

This Indian Standard was adopted by the Bureau of Indian Standards, after the draft finalized by the Food Additives Sectional Committee had been approved by the Food and Agriculture Division Council.

Aspartame is a non-nutritive sweetener, sugar substitute and flavour enhancer. It is 100 to 200 times sweeter than sucrose and has an acceptable dietary intake of 0-40 mg/kg body weight. It is normally used by diabetics. It has been permitted for use under the PFA Rules. It is not recommended for use by pregnant women and phenylketonurics.

Chemical Names and Formulae

The recognized chemical names are N-L-α-Aspartyl-L-phenylalinine-1-methyl ester (APM) and 3-amino-N-(α-carbomethoxy-phenethyl)-succinamic acid. Its empirical formula is $C_{14} H_{18} N_2O_5$, molecular weight 294·31 and structural formula as under :



In the preparation of this standard, considerable assistance has been derived from the following publications:

FAO Food and Nutrition Papers No. 19 'Specification for identity and purity of carrier solvents, emulsifiers and stabilizers, enzyme preparations, flavouring agents, food colours, sweetening agents and other food additives, published by the Joint FAO/WHO Expert Committee on Food Additives, Rome, 1981.

Food Chemical Codex, 1981, published by National Academy of Sciences, National Research Council, Washington DC, USA.

For the purpose of deciding whether a particular requirement of this standard is complied with, the final value, observed or calculated, expressing the result of a test or analysis, shall be rounded off in accordance with IS 2 : 1960 'Rules for rounding off numerical values (*revised*)'. The number of significant places retained in the rounded off value should be the same as that of the specified value in this standard.

Indian Standard
ASPARTAME, FOOD GRADE — SPECIFICATION

1 SCOPE

1.1 This standard prescribes the requirements and methods of sampling and test for aspartame, food grade.

2 REFERENCES

2.1 The following Indian Standards are necessary adjuncts to this standard:

IS No.	*Title*
1070 : 1992	Reagent grade water (*third revision*)
1699 : 1974	Methods for sampling and test for food colours (*first revision*)
2362 : 1973	Determination of water by the Karl Fischer method (*first revision*)
2557 : 1963	Annatto colour for food products

3 REQUIREMENTS

3.1 Description

The material shall be a white, odourless, crystalline powder, having a strong, sweet taste.

3.2 Identification Test

3.2.1 *Solubility*

The material shall be slightly soluble in water and ethanol/methanol.

3.2.2 *Positive Test for Amino Group*

Dissolve 2 g of ninhydrin in 75 ml of dimethylsulfoxide. Add 62 mg of hydrindantin, dilute to 100 ml with 4 *M* lithium acetate buffer solution (*p*H 9), and filter. Transfer about 10 mg of the sample to a test tube, add 2 ml of the reagent solution, and heat. A dark purple colour is formed.

3.2.3 *Positive Test for Ester Group*

Dissolve about 20 mg in 1 ml of methanol. Add 0.5 ml of methanol saturated with hydroxylamine hydrochloride, mix, and then add 0.3 ml of 5 N potassium hydroxide in methanol. Heat the mixture to boiling, then cool, adjust the *p*H to between 1 and 1.5 with hydrochloric acid (ten percent), and add 0.1 ml of ferric chloride (9 g $Fecl_3$. $6H_2O$ in water to make 100 ml). A burgundy colour is produced.

3.3 Specific Rotation

$[\alpha]_D^{20}$: Between + 14.5° and + 16.5° calculated on dry basis when determined in a solution containing 4 g of sample in sufficient 15 N formic acid to make 100 ml and determined within 30 min after preparation of the sample solution.

3.4 The transmittance of a 1-percent solution in 2 N hydrochloric acid, determined in a 1-cm cell at 430 nm, with a suitable spectrophotometer, using 2 N hydrochloric acid as a reference, is not less than 0.95, equivalent to an absorbance of not more than approximately 0.022.

3.5 The material shall also conform to the requirement given in Table 1:

Table 1 Requirements for Aspartame, Food Grade
(*Clause* 3.5)

Sl No.	Characteristic	Requirements	Method of Test, Ref to	
			Annex of this Standard	Other Standard
(1)	(2)	(3)	(4)	(5)
i)	Purity as $C_{14}H_{18}N_2O_5$, percent by mass (on dry basis)	98.0 - 102.0	A-1	—
ii)	Moisture, percent by mass, *Max*	4.3	A-2	—
iii)	Sulphated ash, percent by mass (on dry basis), *Max*	0.2	A-3	—
iv)	*p*H (0.8 percent solution)	4.5 - 6.0	—	—
v)	5-Benzyl-3, 6-dioxo-2-piperazineacetic acid, (diketopiperazine), percent by mass, *Max*	1.0	A-4	—
vi)	Heavy metals (as Pb), mg/kg, *Max*	10	A-5	—
vii)	Arsenic (as As), mg/kg, *Max*	3	—	9 of IS 1699 : 1974

4 PACKING, STORAGE AND MARKING

4.1 Packing

The material shall be filled in containers with as little air space as possible. The containers shall be such as to preclude contamination of the contents with metals or other impurities.

4.2 Storage

The material shall be stored in a cool and dry place so as to avoid excessive exposure to heat.

4.3 Marking

Each container shall be marked legibly to give the following information:

a) Name of the material including the words 'Food Grade';
b) Source of manufacture;
c) Minimum net mass;
d) Batch or code number;
e) Date of manufacture;
f) Date of expiry (not more than 3 years from the date of packing);
g) WARNING
  - Artificial sweetener for diabetics;
  - Not to be used by pregnant women and phenylketonurics; and
h) Any other details required under the *Standards of Weights and Measures (Packaged Commodities) Rules, 1977/Prevention of Food Adulteration Rules, 1955.*

4.4 Standard Marking

The container may also be marked with the Standard Mark.

5 SAMPLING

5.1 Representative samples of the material shall be drawn and conformity of the material to the requirements of this specification shall be determined according to the method prescribed in Annex A of IS 2557:1963.

6 TESTS

Tests shall be carried out by the methods specified in **3.2**, **3.3**, **3.4** and col 4 and 5 of Table 1.

6.1 Quality of Reagents

Unless specified otherwise, pure chemicals and distilled water (*see* IS 1070 : 1992) shall be employed for tests.

NOTE — 'Pure chemicals' shall mean chemicals that do not contain impurities which affect the results of analysis.

ANNEX A

(*Table* 1)

METHODS OF TEST FOR ASPARTAME, FOOD GRADE

A-1 PURITY

A-1.1 Reagents

A-1.1.1 *Dimethylformamide*

A-1.1.2 *Thymol Blue*

A-1.1.3 *Lithium Methoxide/Sodium Methoxide*

A-1.2 Procedure

Weigh accurately about 150 mg of the sample, previously dried at 105° for 4 hours. Dissolve in 35 ml of dimethylformamide. Add 5 drops of thymol blue and titrate with a microburette to a dark blue end-point with 0.1 *M* lithium methoxide. Perform a blank determination and make any necessary correction. Each ml of 0.1 *M* lithium methoxide is equivalent to 29.43 mg of $C_{14}H_{18}N_2O_5$.

NOTES

1 Protect the solution from absorption of carbon dioxide and moisture by covering the titration used with aluminium foil while dissolving the sample.

2 In case lithium methoxide is not available, sodium methoxide may be used for the titration.

A-2 DETERMINATION OF MOISTURE

A-2.0 For routine purposes, moisture shall be determined by the oven method given below. In case of dispute moisture shall be determined by the Karl Fisher Method described in IS 2362 : 1973.

A-2.1 Apparatus

A-2.1.1 *Oven* — electric, maintained at 105° ± 1°C.

A-2.1.2 *Weighing Bottle* — Glass-stoppered, shallow.

A-2.2 Procedure

A-2.2.1 Weigh accurately about 10 g of the well-mixed material in the tared weighing bottle. Distribute the material as evenly as practicable to a depth of about 5 mm. Place the bottle containing the material (uncovered) in the oven maintained at 105° ± 1°C. Remove the bottle from the oven after four hours, close the bottle promptly and allow it to come to room temperature in a desiccator. Weigh it.

A-2.2.2 *Calculation*

$$\text{Moisture, percent by mass} = \frac{100 \times (M_1 - M_2)}{(M_1 - M)}$$

where

M_1 = mass, in g, of the bottle with the material before drying;

M_2 = mass, in g, of the bottle with the material after drying and after it has come to room temperature; and

M = Mass, in g, of the empty bottle.

A-3 DETERMINATION OF SULPHATED ASH

A-3.1 Reagents

A-3.1.1 *Dilute Sulphuric Acid* — 10 percent (*m*/*v*).

A-3.2 Procedure

Transfer about 2 g of the sample, accurately weighed, to a tared 50-ml to 100-ml platinum dish or other suitable container and add sufficient dilute sulphuric acid to moisten the entire samples. Heat gently, until the sample is dry and thoroughly charred, then continue heating until all the sample has been volatilized or nearly all of the carbon has been oxidized. Cool, moisten the residue with 0.1 ml of sulphuric acid, and heat in the same manner until the remainder of the sample and any excess sulphuric acid have been volatilized. Finally ignite in a muffle furnace at 800 ± 25°C for 15 minutes. Cool in a desiccator and weigh.

A-3.3 Calculation

$$\text{Sulphated ash, percent by mass} = \frac{M_1}{M} \times 100$$

where

M_1 = mass, in g, of residue after igniting, and

M = mass, in g, of the material taken for the test.

A-4 DETERMINATION OF 5-BENZYL-3, 6-DIOXO-2-PIPERAZINEACETIC ACID

A-4.1 Apparatus

A-4.1.1 *Gas Chromatograph*

Of a suitable type, equipped with a hydrogen flame ionization detector and designed for handling glass columns with on-column injection (Micro-Tek 220 or equivalent), containing a 1.83 metre x 4 mm (inside diameter) glass column packed with 3 percent OV-1 on 80/100-mesh Supelcoport. Condition the column overnight at 250°C before readjustment and equilibration to the operating condition. To preclude build-up of silicon oxide, clean the detector with acetone frequently.

A-4.1.1.1 *Operating conditions*

The operating parameters may vary depending upon the particular instrument used, but a suitable chromatogram may be obtained using the following conditions:

a)	Column temperature	200°C
b)	Inlet temperature	200°C
c)	Detector temperature	275°C

d) Carrier gas — nitrogen, flowing at a rate of 75 ml per minute

e) Hydrogen and air flow to burner — Optimized to give maximum sensitivity

f) Recorder — 1 mv full scale

A-4.2 Reagents

A-4.2.1 *Silation Reagent*

Just before use, dilute 3 parts; by volume, of N, O-bis-(trimethylsilyl) acetamide with 2 parts of dimethylformamide.

A-4.2.2 *Preparation of Standard Solution*

Transfer about 25 mg of 5-Benzyl-3, 6-dioxo-2-piperazineacetic acid Reference Standard, accurately weighed, into a 50-ml volumetric flask, dissolve in methanol, dilute to volume with methanol, and mix. Pipette 10 ml of this solution into a second 100-ml volumetric flask, dilute to volume with methanol, and mix. Pipette 3-ml of the second solution into a 2-dram vial, with Teflon-lined cap, and evaporate to dryness on a steam bath. Add 1 ml of the Silylation reagent to the residue, cap the vial tightly, shake and heat in an oven at 80° for 30 minutes. Remove the vial from the oven, shake for 15 seconds, and cool to room temperature.

A-4.2.3 *Preparation of Sample Solution*

Transfer about 10 mg of the aspartame sample, accurately weighed, into a 2-dram vial, with Teflon-lined cap, add 1 ml of the Silation reagent, cap tightly, shake, and heat in an oven at 80° C for 30 min. Remove the vial from the oven, shake for 15 seconds, and cool to room temperature.

A-4.3 Procedure

Inject a 3-µl portion of the Standard preparation into the gas chromatograph, obtain the chromatogram, measure the height of the peak produced by the 5-benzyl-3, 6-dioxo-2-piperazineacetic acid, and record it as *P*. Under the stated conditions, the elution time is about 7-9 min. Similarly, inject a 3-µl portion of the sample preparation, obtain the chromatogram, measure the height of the peak produced by the 5-benzyl-3, 6-dioxo-2-piperazineacetic acid contained in the sample, and record it as *p*.

A-4.4 Calculation

$$\begin{array}{l}\text{5-benzyl-3, 6-dioxo-}\\ \quad\text{2-piperazineacetic acid,}\\ \quad\text{percent by mass}\end{array} = \frac{3 \times M \times p}{500 \times m \times p} \times 100$$

where

M = the exact mass, in mg, of the reference standard taken;

m = the exact mass, in mg, of aspartame taken;

p = height of peak produced by 5-benzyl-3, 6-dioxo-2- piperazineacetic acid contained in sample; and

P = height of peak produced by 5-benzyl-3, 6-dioxo-2- piperazineacetic acid contained in standard.

A-5 HEAVY METALS

A-5.1 Reagents

A-5.1.1 *Ammonia Solution*

Dilute 400 ml of 28 percent ammonium hydroxide to 1 000 ml with water.

A-5.1.2 *Acetic Acid Solution*

6 percent (*m*/*v*).

A-5.1.3 *Hydrochloric Acid*

10 percent.

A-5.1.4 *Lead Nitrate Stock Solution*

Dissolve 159.8 mg of lead nitrate Pb $(NO_3)_2$ in 100 ml of water containing 1 ml of nitric acid, then dilute with water to 1 000 ml and mix. This solution should be prepared and stored in glass containers which are free from lead salts.

A-5.1.5 *Standard Lead Solution*

On the day of use, dilute 10 ml of lead nitrate stock solution, accurately measured, with water to 100 ml. Each ml of the solution so prepared contains the equivalent of 10 mg of lead ion (Pb).

A-5.1.6 *Hydrogen Sulphide*

A saturated solution of hydrogen sulphide made by passing H_2S in cold water.

A-5.2 Procedure

A-5.2.1 *Solution A*

Pipette 2.0 ml of standard lead solution (20 µg of lead) into a 50-ml colour comparison tube, and add water to make 25 ml. Adjust the *p*H to between 3.0 and 4.0 (using short range *p*H indicator paper), by addition of diluted acetic acid or ammonia solutions. Dilute with water to 40 ml and mix.

A-5.2.2 *Solution B*

Take 2 g of the sample, accurately weighed in a suitable crucible. Add sufficient sulphuric acid to wet the sample, and carefully ignite at a low temperature until thoroughly charred covering the crucible loosely with a suitable lid during the ignition. After the carbon is thoroughly carbonized, add 2 ml of nitric acid and 5 drops of sulphuric acid, cautiously heat until white fumes are evolved, then ignite, preferably in a muffle furnace at 500° to 600°C until all the carbon is burned off. Cool, add 4 ml of dilute hydrochloric acid (1 in 2), cover, and digest on a steam bath for 10 to 15 minutes. Uncover, and slowly evaporate on a steam bath to dryness. Moisten the residue with 1 drop of hydrochloric acid, add 10 ml of hot water, and digest for 2 minutes. Add ammonia test solution dropwise until the solution is just alkaline to litmus paper, dilute with water to 25 ml and adjust the *p*H to between 3.0 and 4.0 (using short range *p*H indicator paper) by addition of diluted acetic acid solution. Filter, if necessary. Rinse the crucible and the filter with 10 ml of water, transfer the solution and rinsings to a 50-ml colour comparison tube. Dilute with water to 40 ml and mix.

To each tube add 10 ml of freshly prepared hydrogen sulphide test solution. Mix and allow to stand for 5 minutes and view downward over a white surface. The colour of solution B shall not be darker than that of solution A.

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